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ANTITUMOR AND IMMUNOLOGICAL ACTIVITY OF LENTINAN IN COMPARISON WITH LPS

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Abstract — Lentinan manifests marked antitumor and antimetastatic activity in numerous tumor/host systems, and prevents chemical and viral carcinogenesis. Modulation of immune or vascular functions by lentinan is involved in its antitumor effects. The impact of lentinan on the functions of macrophages is distinct from that of LPS. One of the effects of lentinan on the vascular system is the vascular dilatation and hemorrhage (VDH) reaction, and the effect can be monitored as augmented skin reactions to vasoactive mediators. Lentinan induces the VDH-like reaction at the tumor site, resulting in the induction of hemorrhagic necrosis and complete regression of the tumor. In contrast to LPS-induced tumor necrosis (Shwartzman's-like reaction), lentinan-induced tumor necrosis is T-cell dependent.

It has been widely accepted that the host defense immune systems play substantial roles in the therapeutic outcome of anticancer drugs (Burnet, 1970). Immunotherapy using biological response modifiers (BRMs) aims at controlling tumor growth by the augmentation of the host defense/immune systems. Lentinan, a fully purified polysaccharide obtained from *Lentinus edodes* (Berk.) Sing., an edible mushroom, is a true host defense potentiator in the sense that it lacks direct cytotoxic effects against tumor cells and that its antitumor effects are mediated through the host defense/immune systems (Hamuro & Chihara, 1984). Previous studies have demonstrated that lentinan manifests a marked antitumor activity in allogeneic, syngeneic and autochthonous primary hosts, and prevents chemical and viral carcinogenesis (Chihara, Maeda, Hamuro, Sasaki & Fukuoka, 1969; Zakany, Chihara & Fachet, 1980; Suga, Shiio, Maeda & Chihara, 1984). Lentinan is also effective against bacterial, viral, and parasitic infections (Chihara, 1992).

The mode of action underlying the antitumor effects of lentinan has been clarified by recent investigations (Hamuro & Chihara, 1984; Suzuki, Kikuchi, Takatsuki & Hamuro, 1994). Augmented induction by lentinan of cytokines, immune effector cells and vascular responses at local tumor sites are supposed to be involved in the expression of antitumor effects of lentinan.

This article discusses the antitumor and immunological activities of lentinan in comparison with those of LPS, with emphasis on local inflammatory-stromal reactions at tumor sites.

ANTITUMOR ACTIVITIES OF LENTINAN

Previous studies have confirmed that lentinan exerts prominent antitumor effects on various syngeneic and autochthonous tumors as well as on allogeneic tumors. Complete tumor regression and prolongation of survival by the treatment of lentinan alone was observed against not only allogeneic S180 sarcoma but also syngeneic A/Ph. MC.S1 sarcoma, DBA/2.MC.CS-1 fibrosarcoma, MM46 mammary tumor, MH134 hepatoma, and FBL-3 erythroleukemia (Hamuro & Chihara, 1984; Zakany *et al.*, 1980; Suga *et al.*, 1984; Suzuki, Kikuchi, Takatsuki & Hamuro, 1993).

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Table 1. Both tumor cellular reaction and auto CTL activation are required for antitumor effects

Tumor system		Treatment	Local reaction (vascular permeability /infiltration of host cells)	IL-2 responsiveness	IL-2 production	Auto killer activity	Antitumor effects
Type-I	FBL-3	–	–	+ +	+ +	+ +	–
		LNT	+ +	+ +	+ +	+ +	+ + +
Type-II	S908.D2	–	–	–	+ +	–	–
		LNT	–	+ +	+ +	+ +	+
		5-FU/LNT	+ +	+ +	+ +	+ +	+ + +
	MM102	–	–	–	+ +	–	–
		LNT	–	+ +	+ +	+ +	+
		radiation/LNT	+ +	+ +	+ +	+ +	+ + +
Type-III	MBL-2	–	–	–	–	–	–
		LNT	+	+ +	–	–	+
		IL-2	–	–	+ +	+	+
		LNT/IL-2	+ +	+ +	+ +	+ +	+ + +
Type-IV	MM46	–	–	+ +	–	–	–
		CY	+ +	+ +	–	–	+
		CY/IL-2	+ +	+ +	+ +	+ +	– + +

Antitumor effects: –, no effect; +, >70%; + + +, complete regression.

Lentinan exerted synergistic antitumor effects when combined with various therapies, such as chemotherapy, cytokine treatment, and radiation, even against tumors resistant to lentinan alone. Shiio *et al.* demonstrated that the combination of radiotherapy with lentinan augmented survival ratio up to 50% in MM102/C3H system, while lentinan treatment alone showed almost no effect (Shiio *et al.*, 1988). Recently, we observed that low dose chemotherapeutics which have only marginal temporal growth inhibitory effects markedly augmented antitumor effects of lentinan in S908.D2 fibrosarcoma/B10.D2 system (Kikuchi, Suzuki, Miyasaka, Takatsuki & Hamuro, 1991). While none of ten mice treated with 5-Fu (50 mg/kg, day 10) and four of ten mice treated with lentinan (5 mg/kg, day 17 – 21) alone could induce complete regression of tumors, the combination therapy induced complete regression of tumors and complete survival in all mice (ten of ten). Furthermore, prominent synergism between lentinan and IL-2 was also observed in several syngeneic tumors (Suzuki *et al.*, 1994).

Characteristic aspects of antitumor effects of lentinan are the existence of optimal dose and timing of administration to induce its optimal inhibitory effects (Hamuro & Chihara, 1984), as is the case for LPS (Berendt, North & Kirstein, 1978). Decreased antitumor effects by high doses of lentinan are not due to the toxic side-effects. The existence of optimal timing of administration in expressing the antitumor effects has also been observed for LPS (Berendt, North & Kirstein, 1978).

Successful immunotherapy requires the activation of antitumor effector cells in cancer patients. For effective activation of antitumor effector cells the following three steps are required, namely, antigen recognition, expression of lymphokine receptors on the pre-effector cells, and production of lymphokines from lymphokine-producer cells. However, in cancer patients the suppression of lymphokine production and of responsiveness to lymphokines has been frequently observed. Furthermore, recognition of antigens by lymphocyte needs processing of antigens by antigen processing cells, such as macrophages (Rosenthal & Shevach, 1973). Therefore partial destruction of tumor cells by non-specific effector cells, such as neutrophils or macrophages (local reactions) is the prerequisite to initiate antigen-specific immune reactions. Lentinan has shown the ability to enhance the responsiveness of antitumor effector cells to lymphokines (Hamuro & Chihara, 1984). These basic findings naturally lead us to postulate that the therapeutic efficacy of lentinan among different host–tumor systems may be variable, depending on the interaction of tumor cells and host immune cells either surrounding or infiltrating the tumor site (Table 1). In the tumor–host system (FBL-3, S180, MH134, etc.)

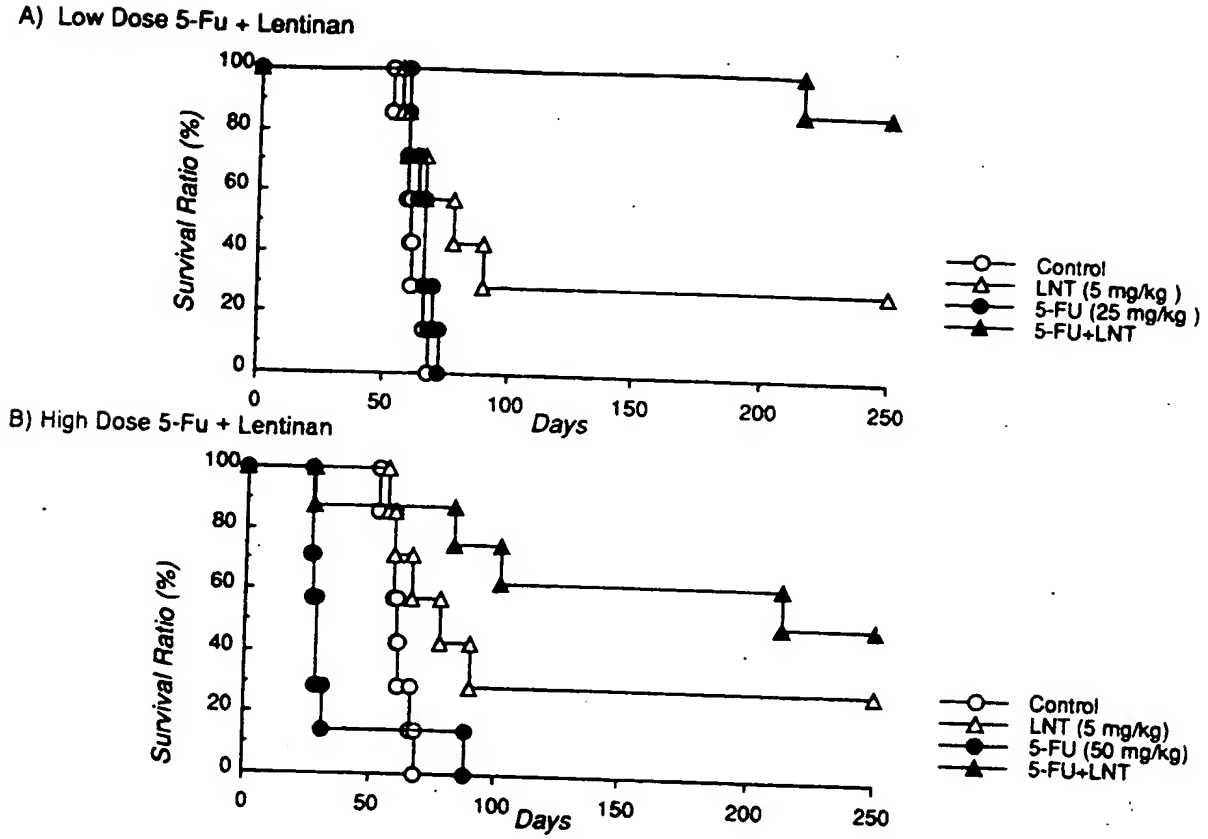

Fig. 1. Curative effectiveness of the combination of lentinan and a low-dose chemotherapy for B10.D2 mice bearing a S908.D2 fibrosarcoma. Samples containing 2×10^6 cells of S908.D2 tumor were transplanted i.d. on day 0. Survival curves of S908.D2-bearing B10.D2 mice treated with saline (on days 10, 12, 14, 17–21) (○), mice treated with 5-Fu (A, 25 mg/kg; B, 50 mg/kg; i.p on days 10, 12, 14) (●), mice treated with lentinan (5 mg/kg, i.p. on days 17–21) (△), and mice treated with both 5-Fu and lentinan (▲).

where responsiveness to lymphokines was suppressed, lentinan alone exerted prominent antitumor effects. In contrast, in the tumor–host system where local cellular reactions and responsiveness to lymphokines were suppressed, lentinan alone did not exert antitumor effects, and combination with chemotherapy (S908.D2) or radiotherapy (MM102) was required to initiate local cellular reactions. Combined chemotherapy and radiotherapy can initiate local cellular reactions; however, intensive chemotherapy or radiotherapy often induce immunosuppression, thereby canceling the effect of cellular reactions they initiated. The combination of low dose chemotherapy and lentinan exerted more prominent antitumor effects than the combination of intensive chemotherapy and lentinan as illustrated in the S908.D2/B10.D2 system (Fig. 1). In the tumor–host system where both lymphokine production and responsiveness to lymphokines were suppressed (MBL-2), the combination of lymphokines and lentinan was required to express fully its antitumor effects. We found that the combination of lentinan with IL-2 induced synergistic augmentation of tumor-specific CTL activity (killing activity: lentinan alone, 3.9%; IL-2 alone, 14.6%; combination, 24.3%) resulting in synergistic antitumor effects (survival ratio: lentinan alone, 0/9; IL-2 alone, 0/9; the combination, 8/9) in MBL-2/BDF1 system (Suzuki *et al.*, 1994). Synergism between lentinan and IL-2 was also observed in growth inhibition and prolongation of survival in the S908.D2 fibrosarcoma/B10.D2 system (Suzuki *et al.*, 1994).

One of the most important and promising areas of BRM application is the prevention of recurrence and metastasis. Anti-metastatic activity of lentinan is observed by both pre-operative and post-operative administration. In the MH134/C3H system, 1 mg/kg of lentinan injected in mice 2–14 days after foot amputation prevented relapse by post-operative metastasis by 100%, while about 70% of mice receiving only foot amputation died by

mestastasis within 8 weeks after operation. Furthermore, pre-operative administration (1 mg/kg, day 7 – 17 after tumor inoculation) of lentinan to mice carrying DBA/2.MC.CS-T fibrosarcoma inhibits over 90% of colony generation in lung after foot amputation (Suga, Yoshihama, Tsuchiya, Shiio, Maeda & Chihara, 1989). The efficacy of preoperative administration of lentinan was also confirmed in the Madison lung 109 carcinoma systems (Rose, Reed, Siminoff & Bradner, 1984). By histological studies of regional lymph nodes at the time of operation, it was confirmed that pre-operative administration greatly prevented the regional lymph node metastasis (Suga *et al.*, personal communications).

Recently, we found that the combination of pre-operative and post-operative therapy using lentinan and IL-2 exerted synergistic curative effects on DBA/2.C.CS-T fibrosarcoma, and the effects were abolished by the treatment of both mAb against CD4 and CD8. Furthermore, cured mice acquired tumor-specific memory (Kikuchi, Suzuki, Suga, Namiki, Takatsuki & Hamuro, 1992). The putative mechanisms of synergistic antimetastatic effects of LNT/IL-2 therapy are as follows: (1) pre-operative treatment of the combination triggers partial destruction of tumors at the original tumor site, and induces tumor antigen-specific memory cells; (2) post-operative treatment induces the clonal expansion and activation of tumor antigen-specific memory cells; (3) activated memory cells exhibit antimetastatic effects against established ecotopic micrometastasis. The acquisition of tumor antigen-specific memory is the most relevant feature for the prevention of metastasis.

IMMUNOLOGICAL ACTIVITIES OF LENTINAN

The importance of cellular immunity mediated by T-lymphocytes in the expression of antitumor effects of lentinan was confirmed using nude or neonatally thymectomized mice (Maeda, Hamuro & Chihara, 1971). Lentinan augments the generation of antigen-specific cytotoxic T-lymphocytes (CTLs) *in vivo* and *in vitro*, through augmentation of CTLs' responsiveness to IL-2 (Hamuro *et al.*, 1979; Hamuro, Wagner & Rollinghoff, 1978). Augmentation of responsiveness to IL-2 by lentinan was also observed in NK and LAK cell activation (Suzuki, Higuchi, Taki, Taki, Miwa & Hamuro, 1990). In contrast to LPS, lentinan did not augment NK and LAK activities *in vitro* (Suzuki *et al.*, 1990). Lentinan augmented the reactivity of macrophages to macrophage-activating factor (MAF) (Hamuro & Chihara, 1984). Although LPS is able to induce activation of macrophages *in vitro*, lentinan-stimulated macrophages were neither cytotoxic nor cytostatic, but they possessed elevated reactivity to MAF (Hamuro & Chihara, 1984). Furthermore, augmentation of the reactivity of macrophages to MAF was also observed in immunocompromised mice accompanied with the tumor growth. The response of macrophages in L5178Y-bearing mice was significantly suppressed when compared with that in normal mice on day 25; only 8% target cell growth inhibition in the presence of MAF by the macrophages from tumor-bearing mice, while 30.4% inhibition from normal mice. However, lentinan treatment of these L5178Y-bearing mice resulted in augmented response to 74.6%. Similar augmentations of responsiveness of CTL, NK, and LAK to IL-2 in immunocompromised tumor-bearing mice have been also confirmed (Hamuro & Chihara, 1984; Suzuki *et al.*, 1990).

Lentinan treatment *in vivo* also enhances the ability of macrophages to produce both IL-1 and IL-6 after *in vitro* culture. The augmented production of IL-1 and IL-6 may also be involved in augmenting responsiveness of CTLs to IL-2, because these monokines possess the ability to induce IL-2 receptors on T-cells (Mannel, Mizel, Diamanstein & Falk, 1985).

Lentinan also possesses effects on the immune regulatory macrophages. Recently, we found that multiple injection of lentinan continuously induced a low but significant level of IL-6 production in serum (Miyasaka, Takatsuki, Suzuki, Kikuchi & Hamuro, 1991). The production kinetics and the amount of IL-6 induced by lentinan were clearly different from those induced by LPS. Although 500 μg/kg LPS induced around one-hundred ng/ml IL-6 with the peak 2 h after injection, lentinan (10 mg/kg × 3 times) induced only around several hundred pg/ml IL-6 after 3 – 7 days of the final injection. It is well known that lentinan induces certain types of acute phase proteins (APPs), such as haptoglobin, hemopexin, and ceruloplasmin, and that the induction of APPs by lentinan is under genetic control (Maeda, Sakaizumi, Moriwaki, Chihara & Yonekawa, 1992a). Lentinan-treated mice were divided into two distinct phenotypic groups: in one, sensitive strains such as DBA/2, C57BL/6, SWR/L or CDF-1 showed a marked increase in the levels of APPs, and in the other resistant strains such as A/J, C3H/HeN, C3H/HeJ, BALB/c, MA/MyJ, or AKR/J mice showed no increase of APPs. Analysis of genetic control of the APPs production by

lentinan using F1 hybrid and H-2 congenic mice revealed that the trait of the APP production by lentinan is controlled under a single major gene, designated *ltn-1*, on an autosome, which has a recessive phenotype. *ltn-1* phenotype does not correlate with the H-2 complex (Maeda, Sakaizumi, Moriwaki & Yonekawa, 1992b). *ltn-1* gene is also different from *Lps* gene, because both C3H/HeN, which shows LPS^n phenotype (LPS-responder) and C3H/HeJ, which shows LPS^d (LPS-low responder) (Morrison & Ryan, 1979), are low responders for lentinan induced-APP production. Since IL-6 has been known to be a potent inducer of APPs, IL-6 production by lentinan seems to be involved in the induction of APPs by lentinan. Recently, we found that the phenotype of the IL-6 production induced by lentinan correlates with the phenotype of *ltn-1*, and that IL-6 infusion in resistant strain mice could induce APPs in serum (Miyasaka *et al.*, 1991). These results suggest that *ltn-1* gene controls the phenotype of IL-6 induction by lentinan, rather than the phenotype of APP induction by IL-6 (or lentinan).

Lentinan is also known to affect the vascular system (Maeda, Watanabe, Chihara & Rokutanda, 1984). The effect of lentinan on the vascular system is known as the vascular dilation and hemorrhage (VDH) reaction. VDH reaction has the aspect with marked dilatation and subsequent hemorrhage of the venule in very localized areas such as ears, feet and tail, peaked on the 4th day after an i.p. injection of lentinan in normal mice. VDH reaction induced by lentinan is also controlled under a single dominant gene, designated *Ltn-2*, which is different from both *ltn-1* and LPS^d gene (Maeda *et al.*, 1992b). In contrast with Shwartzman's reaction which is the most typical vascular reaction induced by LPS, lentinan-induced VDH reaction is fully T-cell dependent (Maeda *et al.*, 1992b).

Recently, we found that lentinan augments vascular reactions against vasoactive mediators (skin reaction) (Takatsuki, Namiki, Kikuchi, Suzuki & Hamuro, 1993). Lentinan treatment of mice (5 mg/kg, 5 times), enhances by two- or three-fold the area of extravasation of i.v. injected Evans Blue observed at the i.d. injection sites of 50 μM of bradykinin as compared with that in control mice. Furthermore, it was clarified that these skin reactions induced by lentinan are also T-cell dependent and that the strain difference of the intensities of the skin reactions coincided with those observed in VDH responses. These findings suggest that skin reactions are also controlled by the *Ltn-2* gene. The expression of the antitumor effects of lentinan is also under genetic restriction, as illustrated by the divergent effectiveness of lentinan among different strains in Sarcoma 180 growth inhibition and in the suppression of 3-methylcholanthrene-induced carcinogenesis (Suga *et al.*, 1984).

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